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METHOD FOR IN-VITRO TESTING OF ACTIVE SUBSTANCES, DEVICE, AND ITS USE

[0001] Description:

[0002] The invention relates to a method for testing active substances on cells in-vitro, a device, and its use.

Discussion of Related Art

[0003] Testing active substances such as cytostatics, for example, that can be used in cancer chemotherapy, is necessary for many reasons. A distinction must be made as to whether a completely new active substance is involved, or whether an active substance whose effectiveness is already basically known is involved. In the case of an unknown active substance, the basic effectiveness, for example, within the scope of an active substance screening, and the dose and the optimum application form, for example, oral or intravenous, must be determined first. In the case of already-known active substances, for example, possible therapeutic strategies are tested and compared with one another. Another important issue is the patient-specific testing or patient-specific effects of a substance on an individual patient. For each of these questions different model systems are available.

[0004] For active substance screening of unknown substances, work is performed without exception, not on patients due to ethical reasons, but on animal models in-vivo or in-vitro with cell lines. Animal models for screening unknown cytostatics are based on the fact that for each treatment of the receiving animal human tumor cell samples are encapsulated in hollow fiber devices and implanted in the receiving animal, as described in WO 94/25074 and U.S. Patent No. 5,676,924. This procedure has its limits, however, since animal models are naturally difficult to automate and are associated with great effort and high costs. Increasingly, the idea of animal protection counteracts testing in animal models.

[0005] The transferability of the test results in experimental animals, typically rodents, to man is also a question. An important role is played by metabolic differences between humans and animal species, leading to different pharmacokinetics in animal models. Within the scope of this invention, pharmacokinetics is understood to be the dynamic process by which an active substance is absorbed with different kinetics in the organism, thus absorbed, distributed, metabolized, i.e., reacted and excreted again. These processes cannot be quantified independently of one another in-vivo since they overlap in time and take place in mutual dependence. In the Compendium of Internal Oncology, published by H.J. Schmoll, K. Höffgen, K. Possinger, Springer Verlag (1996), mathematical models were therefore used, which describe the distribution of a medication in theoretical body spaces or compartments. In particular, in addition to active substance properties and metabolism, the form of application acts on the pharmacokinetics of the substance. The pharmacokinetics of an active substance are usually determined in-vivo by measuring the serum concentration as a function of time.

[0006] The use of in-vitro models for testing substances is also widespread. Cultivation of human primary cells in monolayer cultures for patient-specific testing is preferred to achieve a high sample throughput. Such a procedure was described, for example, by G.J.L. Kaspers et al. in Blood (1997), Volume 90, No. 7, pages 2723-29 and by R. Pieters in Blood (1990), Volume 76, No. 11, pages 76, 2327-36. The disadvantage of known in-vitro methods is that the microtiter plates used in them do not allow three-dimensional growth of the cells. Solid tumors, for example, develop different subpopulations probably because of gradients in the pH value and the nutrient supply. These gradients cause regional variations in cell vitality, metabolism, and sensitivity to treatment with cytostatics. It has been suggested that malignant cells

in a three-dimensional tissue-like structure have a different response to cytostatics than monolayer cultures, as follows from J.J. Casciari et al., J. Natl. Cancer Inst. (1994), Volume 86, pages 1846-52 and K.M. Nicholson et al., Ann. Oncology (1996), Volume 7, Supplement 1, Abstract.

[0007] Another disadvantage of known in-vitro methods is that the pharmacokinetics that take place in the human organism cannot be replicated in-vitro as yet, thus they cannot be modeled by corresponding in-vitro pharmacokinetics. However, the scope of the present invention covers in-vitro pharmacokinetics. This means that the active substance concentration in the vicinity of the target cells changes as a function of the time in-vitro in the same way as in the in-vivo environment of the target cells, as in leukemias in the serum, regardless of what means achieve this goal. The primary criterion is, therefore, the shape of the curve and the absolute value of the active substance concentration, while the time axis can be tightened up or stretched out relative to the in-vivo situation.

[0008] A third disadvantage of all of the in-vitro methods known today for active substance testing is that no combination therapy can be simulated. This is, therefore, of critical importance because today only in exceptional cases the treatment is performed with only one cytostatic, i.e., with a monotherapy. So, most therapies are combination therapies, in which a time sequence of different cytostatics takes place, as in the Compendium of Internal Oncology, published by H.J. Schmoll, K. Höffgen, K. Possinger, Springer Verlag (1996). In the standard in-vitro method, an active substance that has been introduced into the assay cannot be removed again as it is possible in the organism before the next active substance is given.

[0009] All three of these disadvantages lead to the fact that currently the response of real tumors to chemotherapy in-vitro cannot be simulated very well. This

leads to serious doubts among experts about the prognostic value of the in-vitro methods known thus far for testing active substances in cells.

[0010] So, there is a need for an in-vitro method for testing active substances on cells and for a device that can be used for this method, in order that the above-mentioned disadvantages can be at least considerably reduced.

SUMMARY OF THE INVENTION

[0011] The present invention, therefore, has the goal of providing an in-vitro method for testing active substances on cells, and a device that can be used for this purpose so that the disadvantages mentioned above can be at least considerably reduced and an approximation to in-vivo pharmacokinetics can be achieved by suitable in-vitro pharmacokinetics, and combination therapy with different active substances can be simulated.

[0012] This goal is achieved by a method for in-vitro testing of active substances on cells comprising at least the following steps:

a) a cell culture container with an interior chamber and an inside wall and with a first and second membrane system located in the interior chamber are made available, with a cell culture space being formed between the membrane systems and the inside wall of the interior chamber;

b) providing a cell culture and a cell culture medium in the cell culture space;

c) adding a fluid nutrient medium to the cell culture space and removing metabolic products by the first membrane system;

d) adding at least one gaseous medium to the cell culture space by the second membrane system;

e) metering at least one active substance to the cell culture space, wherein the addition taking place according to an adjusted active substance concentration-time curve; and

f) monitoring cell vitality.

[0013] The active substances to be tested include those substances within the scope of the present invention whose effect on the cells to be investigated is unknown or is insufficiently known before the test. These can be gaseous active substances such as respiratory poisons or other optionally toxic gases. Among these substances defined above, cytostatics, antibiotics, cytokines, growth factors, or antiviral agents are preferably used. In addition, basically all of the substances as defined above can be tested for their effect on cells when these substances can be added in dissolved form to the cell culture space.

[0014] Cell cultures can be tested using the method of the invention that consist preferably of primary cells or of cell lines, more preferably employing tumor cell lines. The volume of the cell culture space is preferably 0.3 ml minimum and 2.0 ml maximum, and more preferably between 0.5 ml and 1.5 ml. As a result, only a small amount of cell material is used so that, for example, only small amounts of cells need to be removed from a cancer patient for the method of the invention.

[0015] The first membrane system located inside the cell culture container consists of at least one semipermeable membrane, suitable for the addition of a liquid nutrient medium, i.e., a continuous material transport is permitted through the membrane wall by diffusive or convective transport mechanisms. Depending on the requirement, or depending on whether diffusive or convective material transport of the nutrient medium is necessary, nanofiltration, ultrafiltration, or microfiltration membranes are used. For example, dialysis membranes such as CUPROPHAN® or

hydrophilic microporous membranes such as microporous polyethersulfone membranes are used. The second membrane system in the interior of the cell culture container consists of at least one gas transfer membrane, preferably an oxygenation membrane like that commercially available as OXYPHAN®. The membranes of the first and second membrane system are preferably hollow fiber membranes.

[0016] In a preferred embodiment of the method of the invention, a cell culture container comprises the first and second membrane systems made of hollow fibers, which are stacked in multiple layers in the interior chamber.

[0017] The presentation of the cell culture takes place in a preferred embodiment of the method of the invention, when using a cell culture container with a lid by adjusting the cell density in the cell culture medium, opening the lid of the cell culture container, pipetting the desired volume of the cell suspension into the interior of the cell culture container, and closing the cell culture container with the lid.

[0018] In another preferred embodiment of the method of the invention, the cell culture can be introduced through an opening in a side wall of the interior chamber which is provided, for example, with a connection for a syringe, or the like, on the outside of the cell culture container.

[0019] In a third preferred embodiment of the method of the invention, the cell culture can be added through at least one septum through which access to the interior of the cell culture container is possible.

[0020] The cells can be in the form of suspension cells or adhesion-requiring cells, with suspension cells, as a rule, being the cells that come from the blood, and adhesion-requiring cells, as a rule, being those cells that come from the body tissue. The latter can either be placed directly as undivided pieces of tissue in the interior of the cell culture container, or the pieces of tissue are broken up first, and then used as

suspension cells. For adhesion-requiring cells, the interior of the cell culture container is advantageously equipped with a surface to which the cells preferentially adhere. Preferably, this surface consists of protein-coated polycarbonate, or of textile material made of polyester additionally placed in the interior of the cell culture container.

[0021] When the method of the invention is performed, the cell culture container preferably contains at least $1 \cdot 10^5$ cells per ml of cell culture space, more preferably at least $1 \cdot 10^6$ cells per ml of cell culture space. In an especially suitable version, the cell density in the cell culture space is more than $5 \cdot 10^7$ cells per ml of cell culture space. In a preferred embodiment of the method of the invention, suspension cells with a cell density of at least $1 \cdot 10^5$ cells per ml of cell culture space are used. An approximation of the cell density value in the blood is possible in this way. In addition, when using adhesion-requiring cells, the method of the invention preferably comprises a cell density of at least $1 \cdot 10^5$ cells per ml of cell culture space in order to approximate the cell density of body tissue after the corresponding cell growth.

[0022] Advantageously, each cell has an average distance of 0 μm to 600 μm from the closest membrane of the first and second membrane systems. Thereby, the cells are uniformly supplied with nutrient medium and gas. At the same time, metabolic products are carried away uniformly so a state that resembles in-vivo conditions is simulated in a cell culture.

[0023] Media that are usually employed for supplying cells with nutrient substances or for growing cells can be used as the fluid nutrient medium or as the cell culture medium. Preferably, the cell culture medium comprises RPMI 1640. In a more preferred embodiment of the method of the invention, RPMI 1640 and fetal calf serum containing nutrient medium are used.

[0024] A gas mixture is preferred as the gaseous medium having an oxygen partial pressure pO_2 of 0 to 160 mmHg, and a carbon dioxide partial pressure pCO_2 from 0 to 115 mmHg. In a preferred embodiment of the method of the invention, a cell culture medium contains a bicarbonate buffer and the pCO_2 in the introduced gaseous medium is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

[0025] The desired composition of the gaseous medium can be adjusted by the method of the invention in a space where this composition prevails. In this case, the gaseous medium is added through a sterile filter to the second membrane system.

[0026] The second membrane system can also advantageously be used to remove gaseous metabolic products if it is operated in cross-flow mode, and connected with a gas supply line and a gas removal line.

[0027] In one advantageous embodiment of the method, individual active substances and/or combinations of several active substances are added staggered in time. Thus, for example, an individual active substance can be given as a function of time distributed over several sequential doses, or different individual active substances can be added to the cell culture medium in a time-staggered manner. In the same way, a sequence of active substance combinations can be given time-staggered with the active substance combinations remaining the same or changing in their composition. Finally, individual active substances and active substance combinations of the type described above can be given time-staggered in the method of the present invention.

[0028] Active substance administration can be direct or through the first membrane system or, when a gaseous active substance is used, by the second membrane system to the cell culture space. When the active substances are added

through the first membrane system, the active substance is added to the nutrient medium flow and enters the cell culture space with the nutrient medium through the membranes of the first membrane system. When a gaseous active substance is added by the second membrane system, the latter is fed into the gaseous medium and together with the gaseous medium enters the cell culture space through the membranes of the second membrane system. This, of course, means that the membranes of the two membrane systems must be permeable to the respective active substance. When the active substance is administered by means of the first or second membrane system, the active substance concentration-time curve, for example, is adjusted by the permeability of the first or second membrane system, by the duration of the active substance administration, and by the active substance concentration. So, the methods described above allow the simulation of very different pharmacokinetics of individual active substances and/or active substance combinations. For example, the active substance administration, analogous to continuous infusion, can be ramped or analogous to an intravenous administration with peaks.

[0029] During the active substance test, the cell culture container is kept at a temperature suitable for growing the cells, preferably 37°C.

[0030] Monitoring of cell vitality is understood within the scope of the present invention to be the monitoring of the metabolic cell activity, proliferation, apoptosis, or cell death in general.

[0031] To monitor cell vitality, in a preferred embodiment of the method of the invention, a cell vitality dye is used. More preferably Alamar Blue® is used. The cell vitality dye can be administered through the nutrient medium and the first membrane system to the cell culture space. Alamar Blue® penetrates the membranes of the cells, enters the interior of the cell, and is converted by metabolic exchange

there to a fluorescent dye. Therefore, the quantity of fluorescent dye formed can be used as a measure of cell vitality and can be detected by a fluorescence sensor online or after removing a sample in the nutrient medium. It is especially advantageous that removal of a sample from the cell culture space is not necessary, but can take place in the fluid flow leaving the cell culture container. A further advantage is the fact that the cell vitality dye is not only added by the first cell membrane system, but also can be removed completely so that all problems with the cell culture caused by the dye can be corrected and reversed.

[0032] In a method of the invention, at least one sensor can be used to monitor cell vitality, supplying information about the state of the cells. Preferably, these are miniaturized sensors to determine proliferation, vitality, apoptosis, or cell death in general. More preferably the sensor is a sensor for fluorescence.

[0033] In the method according to the invention, suitable sensors can be used to monitor the process. Preferably, sensors for monitoring temperature, pH, partial pressure of oxygen pO_2, or carbon dioxide pCO_2, glucose, or lactate are used. In a more preferred embodiment, the sensors are integrated into the interior of the cell culture container as microsensors, with no disturbing influence of the sensors on the cell culture. In addition, one or more of these sensors can be used in the cell culture space or in the nutrient medium and the sensor signals can be followed online. Thus, for example, pH, pO_2, glucose, and lactate can be measured simultaneously. Instead of, or in addition to the online sensors described above, further preferred analytical methods are used in the method according to the invention for endpoint determination such as MTT assay (mitochondrial reduction of tetrazolium dye) or flow cytometry.

[0034] The goal is also achieved by a device which is used to collect the cell culture in a cell culture medium using a suitable cell culture container with an interior

chamber, wherein first means for adding at least one nutrient medium and second means for adding at least one gaseous medium are located in the interior chamber. The means have a supply side and a removal side and a cell culture space is formed between these means and the inside wall of the interior chamber, the first means with its supply side is linked in a fluid connection by a nutrient medium dispensing unit with at least one nutrient medium container and a second medium is in fluid connection with its supply side by a gas dosing unit with at least one gas supply container. The cell culture space has a volume between 5 ml maximum and 0.1 ml minimum. The device also has an active substance supply container for supplying at least one active substance to the cell culture space, and creating an adjusted concentration-time curve of the active substance in the cell culture space.

[0035] In a preferred embodiment of the device of the invention, the first means are in a fluid connection with a waste container on the outflow side.

[0036] In a further preferred embodiment, the first means are in a fluid connection with the removal side by a recirculation line with the at least one nutrient medium container. In the line between the removal side of the first means and the waste container, a device may be installed that can isolate or detect certain metabolic products.

[0037] The first means located inside the interior of the cell culture container preferably consists of at least one membrane suitable for supplying liquid nutrient media.

[0038] The at least one membrane of the first means preferably comprises a semipermeable membrane suitable for adding a liquid nutrient medium, i.e., continuous material transport through the membrane wall can take place as a result of diffusive or convective transport mechanisms. Depending on the requirements, i.e.,

depending on whether diffusive or convective material transport of the nutrient medium is necessary, a nanofiltration, ultrafiltration, or microfiltration membrane is involved. Dialysis membranes such as CUPROPHAN® or hydrophilic microporous membranes such as microporous polyethersulfone membranes can be used, for example.

[0039] The second means located in the cell culture container preferably comprises at least one membrane suitable for gas exchange.

[0040] The at least one membrane of the second means preferably comprises of an oxygenation membrane, more preferably of at least an OXYPHAN® membrane.

[0041] According to the invention, the second means is in a fluid connection with a gas supply container. The gas supply container may comprise a gas chamber, for example, an incubator and the gas chamber and a second means are linked by a gas-permeable, sterile filter. In a preferred embodiment of the device of the invention, the second means has a fluid connection through at least one gas metering unit with at least one pressurized gas supply container. Different concentrations of various gas components can be adjusted in simple fashion.

[0042] In a preferred embodiment of the device of the invention, the membranes of the first and second means are made of hollow fibers.

[0043] More preferably, the hollow fibers are stacked in several layers in the inner space.

[0044] In another preferred embodiment, the maximum distance between the hollow fibers forming the respective means is between 50 μm and 600 μm.

[0045] In a preferred embodiment, the cell culture container has a bottom and a lid which bound the interior chamber, are located opposite one another, and are

made of a transparent material. The transparency of the bottom and the lid allows microscopic observation of the cells during the tests of the active substance.

[0046] In a more preferred embodiment, a heating system suitable for warming the cell culture container to 37°C is integrated into the bottom, for example, as a heating film which has sufficient transparency for viewing the cell culture, for example, by a microscope.

[0047] The cell culture space preferably has a volume of 0.3 to 3.0 ml. The advantage of this miniaturization consists in especially low use of cells, active substances, liquid, and gaseous media.

[0048] The means preferred in the device of the invention for adding the active substance comprises at least one active substance supply container, at least one active substance metering unit, and a system of pipes that connects the at least one active substance supply container through an active substance metering unit directly or through the first supply device with the cell culture space of the cell culture container.

[0049] In a preferred embodiment of the device according to the invention, the means for creating an active substance concentration-time curve in the cell culture space consists in the permeabilities of the membranes of the first means.

[0050] In a preferred embodiment, the device according to the invention comprises means for monitoring cell vitality.

[0051] A more preferred embodiment of the device of the invention comprises at least one sensor suitable for supplying information about the state of the cell culture as a means for monitoring cell vitality. Preferably, these are miniaturized sensors for determining proliferation, vitality, apoptosis, or generally cell death. More preferably, there is a sensor for detection of fluorescence.

[0052] The device of the invention may contain sensors suitable for monitoring the process, whereby preferably sensors are attached for temperature, pH, partial pressure of oxygen pO_2, or carbon dioxide pCO_2, glucose, or lactate located inside the cell culture container.

[0053] These sensors can be arranged individually or in combinations inside the cell culture container.

[0054] The goal of the present invention is also achieved by using a modular active substance test system comprising at least two of the devices of the invention.

[0055] Preferably, the modular active substance testing system comprises 6, 24 or 96 devices according to the invention which are arranged in a suitable manner to form a modular configuration.

[0056] Finally, the problem of the present invention is solved by a process for in-vitro testing of the effects of active substances on cells comprising the device according to the present invention and the modular active substance testing system according to the invention.

[0055] The device of the invention or the modular active substance testing system may be used to determine the influence pharmacokinetics on cell vitality.

[0056] The device of the invention or the modular effective substance testing system of the invention may be used to determine the influence of pharmacokinetics on the vitality of cells.

[0057] The present invention is explained in greater detail on the basis of the following drawings and the example. The following are shown in a simplified schematic representation:

[0058] Figure 1 is a flowchart of a device of the invention.

[0059] Figure 2a is a cross section through a modular active substance testing system with devices of the invention.

[0060] Figure 2b is a cross section through a modular active substance testing system with three stacked planes with devices of the invention. and

[0061] Figure 3 is a top view of a modular active substance testing system comprising six devices of the invention.

[0062] Figure 4a is an active substance concentration-time curve shown as Profiles 1 to 3.

[0063] Figure 4b shows the vital cells in four different cell culture containers as presented (inoculum) and as harvested cells (cell harvest).

[0064] Figure 1 shows a nutrient medium container 4, connected in a fluid manner by a pipe 14 and a nutrient medium dispensing unit 3 with the supply side of the first means contained in the interior chamber 2 of cell culture container 1. The removal side of the first means located in the interior chamber 2 of cell culture container 1 is connected in a fluid manner by line 14 with a waste container 10. In line 14 between the removal side of the first means and the waste container 10, there is a device 12 which can isolate or detect certain metabolic products. The recirculation line 11 permits a return of the fluid in interior chamber 2 of cell culture container 1 in nutrient medium container 4. An active substance supply container 7 is connected in a fluid manner by a line system 9, an active substance dispensing unit 8, and a line 9b with the supply side of the first means contained in the interior chamber 2 of cell culture container 1. In this configuration, an active substance reaches the interior chamber 2 of cell culture container 1 through the first means. The switching element 9c and line 9a permit a direct fluid connection between an active substance supply container 7 and interior chamber 2 of cell culture container 1. At least one gas

supply container 6 is connected in a fluid manner by a gas dispensing unit 5 with the supply side of the second means located in the interior chamber 2 of cell culture container 1, whose removal side is connected with a gas removal line 6a.

[0065] Figure 2a shows in cross section a modular active substance testing system mounted in a holder 13. A nutrient medium holder 4 is connected in a fluid manner with a nutrient medium line 14 by a nutrient medium dispensing unit 3 made as a tube pump, with the supply side of the first means 1a in the interior of cell culture container 1, with the connection between nutrient medium dispensing unit 3 and the supply side of first means 1a being in the form of lines 14 and 9b. The removal side of the first means 1a in the interior chamber of cell culture container 1 is connected in a fluid manner by a line 14 with waste container 10. However, the removal sides of a plurality of the devices according to the invention can be connected with a common waste container. Cell culture container 1 is placed on a baseplate 15 and fastened there by a latching mechanism. A heating film is integrated into baseplate 15 which is suitable for keeping the cell culture container and the medium at the required temperature, preferably 37°C, shortly before its entrance into the cell culture container. The heating film, however, can also be integrated into the bottom of the cell culture container 1 as long as this integration permits a sufficient transparency of the cell culture-containing bottom which is advantageous for a visual or microscopic observation of the cell culture. In front of and behind cell culture container 1, there are devices 16 suitable for sampling and can be made for example as a septum. The active substance supply container 7 is connected by line system 9, active substance dispensing unit 8, and line 9b with the supply side of first means 1a located in cell culture container 1. The lines carrying the fluids are preferably silicone tubing with an inside diameter of 1 mm.

[0066] Figure 2b shows a modular active substance testing system in cross section with three planes stacked in a holder 13 containing the devices of the invention. Figure 2b shows that the modular design of the devices of the invention provides a plurality of these devices so that many of the active substance tests can be performed simultaneously.

[0067] Figure 3 shows in top view a modular effective substance testing system in a holder 13 that holds six devices of the invention. Five of these devices are identical to the devices described in Figure 2a. The sixth device, shown at the very bottom in Figure 3, is a device 17 suitable for combination therapy, whose three active substance supply containers 18a, 18b, and 18c are connected in a fluid manner with the supply side of the first means by individual active substance pumps 19a, 19b, and 19c. Together with active substance supply container 7 and active substance dispensing unit 8, a combination therapy of four active substances can be performed in the cell culture container of the lowermost device of the invention shown in Figure 3.

[0068] The devices of the modular effective substance testing system isolate nutrient media, cell cultures, active substances, and waste solutions from the outside world. All of the parts of the device that come in contact with the active substance are preferably made as disposable articles. Therefore, each device in the modular system can be removed individually by the above-mentioned latching mechanism from this system. It is not necessary for the operating personnel to then come in contact with the partially highly toxic active substances. All of the parts that come in contact with the nutrient medium, active substance, and cell culture must be sterilizable. Sterile operation of the device for 10 days has been demonstrated.

[0069] Thus, it becomes clear that the modular arrangement of the devices allows a very high number of active substance tests with different pharmacokinetics

and active substance combinations and the possibility of the modular system helps to choose the number and layout of the individual devices in many different ways. For example, reference devices without the addition of active substances can be operated in parallel with devices with active substances. The modular design of the system, consisting of individual devices, has the additional advantage over conventional cell culture vessels (96-wave plates, 24-wave plates, and 6-wave plates) of individual manipulability of the individual devices. However, the same manipulations (same sample, same treatment) can be performed in different channels (multiple measurements).

[0070] A modular effective substance testing system consisting of six devices weighs less than 10 kg and can be easily worn by an individual. Owing to the small dimensions, for example, a modular effective substance testing system consisting of 24 devices can be operated even in a conventional CO_2 incubator.

[0071] A personal computer is usually employed for system control, sample identification, sample collection, and data evaluation, on whose screen the current measured values can be followed. It is also possible to compare data between individual channels. The evaluation software can handle trend analysis and analyze the difference between reference and active substance channels. The results can be evaluated for individual patients and then stored.

[0072] The following example shows how the modular effective substance testing system can be used to measure the influence of pharmacokinetics on the vitality of cells.

[0073] Example:

[0074] The leukemic cell line CCRF CEM was supplied in a density of $1 \cdot 10^7$ per ml of cell culture medium (RPMI 1640 and 10 vol.% fetal calf serum based on

RPMI 1640) and in a volume of 300 µl in four cell culture containers of a modular active substance testing system according to the invention consisting of four devices according to the invention.

[0075] The modular active substance testing system was enclosed in an incubator in which a temperature of 37°C and a gaseous medium consisting of 5% CO_2, 74% N_2, and 21% O_2 was provided. The addition of the gaseous medium just mentioned takes place diffusively using sterile filters via the second membrane system made of OXYPHAN® into the interior of cell culture containers 1 to 4. RPMI 1640 and 10 vol.% of total calf serum based on RPMI 1640 was used as the nutrient medium. For a time of 24 h, the nutrient medium was recirculated at a fluid rate of 7 ml/min, so that the leukemic cell lines were supplied with nutrient medium by the membranes of the first membrane system in the form of hollow fibers made of CUPROPHAN®. After 24 h of recirculation, the nutrient medium supply was interrupted and cytostatic idarubicin was added with three different active substance concentration-time curves with a fluid flow rate of the nutrient medium of 0.2 ml per minute over the CUPROPHAN® membranes in these cell culture containers of the devices according to the invention as described in the following:

[0076] The active substance concentration-time curves are shown in Figure 4a) as Profiles 1 to 3.

[0077] Profile 1: A solution of 0.20 µg of idarubicin per ml of the above-mentioned cell culture medium was fed through the CUPROPHAN® hollow fibers of cell culture container 1 in a period of 75 minutes.

[0078] Profile 2: A solution of 0.50 µg idarubicin per ml of the above-mentioned cell culture medium was conducted for 20 minutes through the hollow

CUPROPHAN® fibers of cell culture container 2. Then a solution of 0.25 μg idarubicin per ml of the above-mentioned cell culture medium was conducted through the hollow CUPROPHAN® fibers of cell culture container 2 for 20 minutes.

[0079] Profile 3: A solution of 1.00 μg idarubicin per ml of the above-mentioned cell culture medium was conducted through the hollow CUPROPHAN® fibers of cell culture container 3 for 15 minutes.

[0080] No idarubicin was added to the interior of cell culture container 4. This cell culture container served as a control. The administration of the active substance took place in such a way that for all of the active substance concentration-time curves described above, the same area under the curve (AUC) resulted. After adding the cytostatic agent the cell culture container was flushed with fresh nutrient medium (RPMI 1640 and 10 vol.% fetal calf serum based on RPMI 1640) for 1 hr, while the nutrient medium was conducted at a flowrate of 0.2 ml/min through the membranes of the first membrane system and the fluid flow emerging from the membranes was conducted into the respective waste containers. Then nutrient medium recirculation was resumed at a flowrate of 7 ml/min. After 72 hr, the previously used nutrient medium was replaced by the same but fresh nutrient medium. After 96 hr, the cells from the four cell culture containers were harvested and the number of vital cells was determined by Trypan Blue dye and as the vitality the percentile component of the number of vital cells harvested to the total number of harvested cells was determined according to the following relationship:

[0081] Vitality = (number of harvested vital cells/total number of harvested cells)·100%.

[0082] In Figure 4b, the word "inoculum" is followed by the number of vital cells supplied in cell culture containers 1 to 4. Since the cells were supplied in a

volume of 300 µl and in a cell density of $1 \cdot 10^7$ per ml of cell culture medium, the number of vital cells supplied in the cell culture containers was 1 to 4 $30 \cdot 10^5$. The words "cell harvest" are followed in Figure 4b by the number of vital cells obtained after the cell harvest. It is clear that the active substance concentration-time curve according to Profile 1 reduced the number of vital cells the most.

[0083] The vitalities of the cells from cell culture containers 1 to 4 were obtained with the following results:

Cell culture container	Vitality
1	29%
2	36%
3	47%
4	90%